



Sullivan
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rivier et al.
Serial No.: 47,026
Filed: June 11, 1979
For: WATER-SOLUBLE PEPTIDES
AFFECTING GONADAL FUNCTION

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D. C. 20231, on this date.

Mne. 27,180
Date
Registration No. 20,856
Attorney for Applicant(s)

Group Art No. 125

Examiner: Delbert T. Phillips

AFFIDAVIT UNDER RULE 132

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APR 11 1980

STATE OF CALIFORNIA)
COUNTY OF SAN DIEGO) SS

GROUP 120

WYLIE W. VALE, JR., being sworn, deposes and says:

(1) THAT he is the Wylie W. Vale, Jr. who is one of the Applicants of the above-identified patent application and that he is presently head of the Peptide Biology Laboratory at The Salk Institute for Biological Studies in San Diego, California, where he is an Associate Research Professor.

(2) THAT he holds a Ph.D. degree in Physiology from Baylor College of Medicine in Houston, Texas, granted in 1968, and has done post-doctoral work in Physiology at Baylor College of Medicine.

(3) THAT he is the co-author of a large number of published articles relating to the synthesis and biological testing of peptides.

(4) THAT he has been informed that the claims of the above-identified application have been rejected as being obvious in view of the disclosure of certain U.S. patents, as well as the disclosure of an article which he co-authored and which was contained in a publication entitled "Peptides 1976".

(5) THAT he is familiar with the article and has reviewed the disclosure of the patents in question and finds no suggestion in them that would lead one to prepare an LRF analog wherein the 6-position is occupied by imidazole benzyl D-His.

(6) THAT out of all of the references cited, only the Wittle et al. patent indicates the possible substitution in the 6-position of D-His (and not imidazole benzyl D-His); however, it is clear that the disclosure of this patent is directed to a nonapeptide wherein the amino acid residue in the 1-position has been deleted and that the aim of the patentees was to stress that the inclusion of any one of a large number of D-isomer amino acids in the 6-position can result in an improvement in the nonapeptide LRF analog.

(7) THAT, aside and apart from the deletion of the amino acid residue in the 1-position, all Wittle et al. disclose with respect to substitutions in the 6-position is the general teaching that can be found in the Peptides 1976 article, namely, that the substitution of certain D-isomers in the 6-position can result in an increase in potency agonists, and in my opinion the article is more pertinent because Wittle et al. were concerned with LRF antagonists.

(8) THAT the present invention is advantageous and unobvious over the prior art for two reasons: namely, (1) the peptides of the invention exhibit hydrophilicity and thus solubility in water greater than that of the LRF analogs

with the D-isomer substitutions in the 6-position disclosed in the references and (2) the peptides of the invention are agonists which exhibit a potency many times greater than LRF and substantially greater than the LRF analogs having such 6-position D-isomer substitutions as are disclosed in the references.

(9) THAT the LRF analog [D-His⁶(imBzl),Pro⁹-NET]-LRF was synthesized, purified and tested as set forth in Example II of the specification and tested in vitro as set forth on pages 17 and 18 of the specification and found to have a potency approximately 217 times as great as LRF.

(10) THAT the following generally comparable LRF analogs, each having a D-isomer substitution in the 6-position and also having the ethylamide modification in lieu of glycine in the 10-position, were synthesized and tested using precisely the same in vitro assay techniques as employed with respect to the testing reported on pages 17 and 18 of the specification, with the following results:

| LRF ANALOG | BIOLOGICAL POTENCY RELATIVE TO LRF (<u>in vitro</u>) |
|--|--|
| [D-Ala ⁶ ,Pro ⁹ -NET]-LRF | 14 |
| [D-Leu ⁶ ,Pro ⁹ -NET]-LRF | 15 |
| [D-Lys ⁶ ,Pro ⁹ -NET]-LRF | 17 |
| [D-Tyr ⁶ ,Pro ⁹ -NET]-LRF | 68 |
| [D-Phe ⁶ ,Pro ⁹ -NET]-LRF | 90 |
| [D-Trp ⁶ ,Pro ⁹ -NET]-LRF | 144 |
| [D-His ⁶ (imBzl),Pro ⁹ -NET]-LRF | 217 |

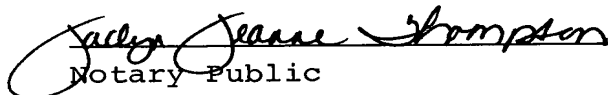
(11) THAT [D-Trp⁶, Pro⁹-NET]-LRF is the most potent analog (next to the peptide of the invention) of those tested, and that, with respect to hydrophilicity, [D-His⁶(imBzl), Pro⁹-NET]-LRF is more than twice as soluble in water under slightly acid conditions at room temperature than is [D-Trp⁶, Pro⁹-NET]-LRF.

(12) THAT in his opinion, the foregoing advantages of higher biological potency and substantially greater solubility render the peptides of the invention nonobvious in view of the disclosure of any of the cited references.


Wylie W. Vale, Jr.

STATE OF CALIFORNIA)
COUNTY OF SAN DIEGO) SS

Sworn to and subscribed by WYLIE W. VALE, JR.
before me this 24th day of March, 1980.


Notary Public

